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Potential molecular targeting of splice variants for cancer treatment

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Abstract

Array of new targets for investigation as cancer therapeutics has great potential to grow as new splice-variants are identified and characterized in cancer cell-lines and tumor samples. Tumor-specific splice variants are being discovered at an increasing rate and their functions are also investigated in cancer progression. The tumor-specific splice variants whose expression patterns and activities are successfully characterized may become attractive targets for ablation or splicing modification. The extreme specificity of their expression suggests that a variant-specific treatment may allow for targeting of cancerous cells with minimal impact to healthy tissues. Clinical investigation of applying antisense oligonucleotides to down-regulate mRNAs that contribute to cancer cell survival and to modify splicing patterns in muscular dystrophy has shown promising results. These results show that antisense therapy may be applied effectively and safely in humans. As these treatment strategies continue to improve and novel tumor-specific splice-variants are identified, modification of splicing patterns will become an important field of investigation to develop more effective and safe cancer therapies.

Keywords

Cancer therapeutics; Expressed sequence tags; Osteopontin-c; SiRNA; Tumour specific splice variants

Alternative splicing

Alternative splicing is the process by which a single gene may produce many different transcripts that can show a wide range of activities, and is responsible for much of the diversity of the human proteome[1]. There also exists a high degree of tissue-specific and condition-specific alternative splicing[2] that creates a virtual guarantee that uncharacterized splice-variants with significant functional differences from the normally expressed version will continue to be discovered, and many of the potential differences such as ligand binding/specificity or effect on cell cycle regulation[3] have strong implications for cancer development and progression. Identification of tumor-specific splice variants is a key first step in the selection of potential novel treatment targets. While direct analysis of tumor samples and cancer cell lines can discover small numbers of new splice variants at a time, large scale sequencing efforts such as the Cancer Genome Project have collected vast amounts of data on cancer genes that, when analyzed, presents potential therapeutic targets

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in the form of genes with characteristic splicing mutations[4]. The usage of expressed sequence tags (ESTs) has allowed the prediction of large numbers of tumor-specific splice variants, however this can only be used as a preliminary means of identification, requiring confirmation of true tumor-specificity by independent methods[5-7]. As more information continues to emerge on the expression patterns of individual splice variants and their implications in cancer development and progression, the promise of new and effective therapeutic targets grows ever stronger.

Role of splice variants in cancer progression

True cancer-specific splice variants present particularly attractive potential targets for therapy when they have been associated with cancer progression, and provide a broadly applicable treatment approach due to the ubiquitous nature of alternative splicing events. Recent report in ovarian cancer has identified tumor-specific splice variants and tested the viability of treatments targeting them, with encouraging results. For example osteopontin-c (OPNc) was found to be expressed in ovarian malignant and borderline tumor samples and cell lines, and was completely absent in healthy tissues. When overexpressed, OPNc, which lacks exon 4 of the full length osteopontin, was observed to dramatically increase cell proliferation, invasion, migration, colony formation, and tumor growth, while other non tumor-specific osteopontin variants did not. This activity suggests that targeted down-regulation of OPNc, which leaves the full-length and non tumor-specific variant expression levels unaltered, may provide a means of preventing tumor progression while leaving healthy tissues unaffected[8]. It was also reported that in taxane-resistant ovarian cancer, siRNA silencing of survivin (an anti-apoptotic protein) variant 2B inhibited both cell growth *in vitro* and tumor growth *in vivo*. Additionally, survivin 2B silencing caused increased sensitivity to docetaxel in taxane-resistant cells, demonstrating that treatment resistance associated with splice variants can potentially be overcome by targeting the variant mRNAs themselves rather than the full length mRNA[9] . Another recently identified splice variant that may develop into a therapeutic target is the coxsackie adenovirus receptor (CAR) variant CAR4/6, which is expressed in cervical cancer samples, but not in normal cervical epithelial tissue. Ectopic expression of CAR4/6 has been found to significantly enhance both proliferation and invasion of multiple cell lines, suggesting that investigation into its targeted down-regulation is warranted and may mitigate the variant's harmful effects[10].

In prostate cancer (PCa), inhibition of androgen receptor (AR) signaling by androgen ablation is a common therapy applied when cancer metastasizes beyond the prostate or recurs after radical prostatectomy[11]. In response to androgen depletion, however, PCa will eventually recur with a castration resistant phenotype (CRPC) that retains its dependence on AR signaling, which the cells are able to maintain despite castrate levels of circulating androgens[12]. One proposed mechanism for progression to CRPC is the significant upregulation of constitutively active AR isoforms lacking the ligand-binding domain present in normal AR[13-17]. The role of splice-variants in CRPC is particularly complex, since evidence seems to indicate that the gain of function granted by the isoforms associated with castration resistance is dependent on the presence of full length androgen receptor molecules, though the exact mechanism of this dependence remains to be determined[18].

Approaches for targeting splice variants

The use of custom designed oligonucleotides to bind to specific target sequences is currently the leading means of correcting conditions associated with pre-mRNA mis-splicing[19]. The potential of applying antisense oligonucleotides (AONs) as a means of splicing modification by inducing the exclusion of aberrant exons has been well established as a viable treatment method, notably in the treatment of Duchenne Muscular Dystrophy (DMD), in which AON-

mediated exon skipping can greatly reduce the severity of the disorder's impact[20-22]. Two phase I/IIa clinical trials for AON-based DMD therapy have shown encouraging results that demonstrate the safety and effectiveness of antisense therapy application in human subjects[23, 24].

Investigation of the concept of using antisense therapy to restore normal cell-cycle regulation in cancer cell lines is becoming a promising approach in mitigating many of the factors that allow cancer development, progression, and survival. A phase II clinical trial is currently underway using antisense inhibition of survivin as a treatment for prostate cancer in combination with docetaxel, after the antisense oligonucleotide (LY2181308) was found to induce apoptosis and also sensitize cells to apoptosis induced by chemotherapy[25]. Inhibiting the expression of full length, fully functional transcripts may, however, be a dangerous approach when the gene also has an important function in healthy cell populations, as may be the case for survivin[26]. However, when a tumor-specific splice variant with activity vital to the cancer cells' survival is identified, it provides an elegant solution to the problem of ablating problematic variant proteins in tumor cells while leaving healthy cells with normal transcripts alone[9].

Another interesting application of antisense therapeutics is the usage of splice switching oligonucleotides (SSOs) to take advantage of the antagonistic effects of the anti-apoptotic Bcl-xL and pro-apoptotic Bcl-xS splice variants of Bcl-x, an apoptotic regulator. By targeting the 5′ splice site of exon 2 of Bcl-x pre-mRNA, the investigators were able to decrease the expression of Bcl-xL and increase expression of Bcl-xS, inducing apoptosis and reducing *in vivo* tumor load[27]. A similar approach may potentially be applied in CRPC if splicing of the AR splice-variant AR-V7, which provides a gain-of-function allowing androgen-independent growth, can be switched to increase the expression of AR-V1, which acts as a dominant-negative inhibitor of AR-V7 (Ref. 18). Use of this approach is likely to be more difficult in the case of AR splicing, however, due to the diversity of AR splice-variants and the recently described detection of intragenic rearrangement and duplication in AR splicing deregulation, which may limit the possibility of manipulating the expression of specific variants[28].

An alternative approach to the inhibition of AR signaling was recently described in which the antibiotic nigericin inhibited both androgen-dependent and androgen-independent growth of AR and truncated AR positive cell lines via destabilization of AR mRNAs and an additional but unidentified post-translational mechanism. The overall effect of nigericin treatment in this study proved to be very similar to the result of siRNA mediated knockdown of full-length and variant transcripts[29]. While this may develop into a promising treatment for CRPC, drugs with similar activities that target both full-length and truncated mRNAs may not be as applicable in cases where the full-length transcript is necessary for vital processes in healthy cells, as in the case of survivin[26]. Additionally, discovering drugs with this sort of activity may not be achieved as reliably as designing an AON or siRNA mediated approach to targeted expression modification simply by nature of the need to screen compounds for activity.

Conclusion

The continuing identification of novel splice-variants in cancerous tissues and cell lines provides a large and rapidly-expanding array of potential therapeutic targets. The steps for identifying novel splice variants as therapeutic targets and approaches for targeting splice variants for cancer are summarized in Figs 1, 2. The expression patterns of many of these variants, once determined, will provide investigators with information about which variants are largely harmless or even beneficial, and which are strongly up-regulated or only

expressed in cancerous cells. While this information alone is valuable as an indicator of a patient's prognosis, more investigation into the *in vivo* and *in vitro* effects of these splice variants may become possible in order to determine which variants result in cell-cycle deregulation or treatment resistance. Such splice variants may be targeted for ablation either as a treatment in itself or to improve the outcomes of existing treatments. The understanding of the role of specific splice-variants in cancer progression improves, an increase in the use of patient-specific expression patterns for treatment decisions and in development of more viable molecularly targeted treatments are expected. While current policy precludes the ability to run clinical trials for all but the most widespread potential targets for alternative-splicing modification, it seems inconceivable that this will remain the case as the body of work demonstrating the efficacy and specificity of splice-variant targeting continues to grow.

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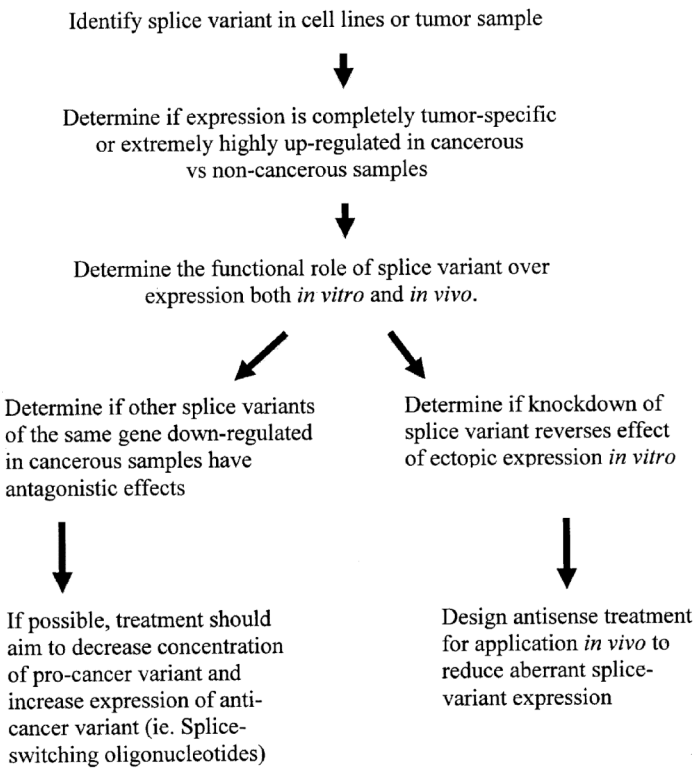

Fig. 1.
Steps to validate splice-variants as treatment targets

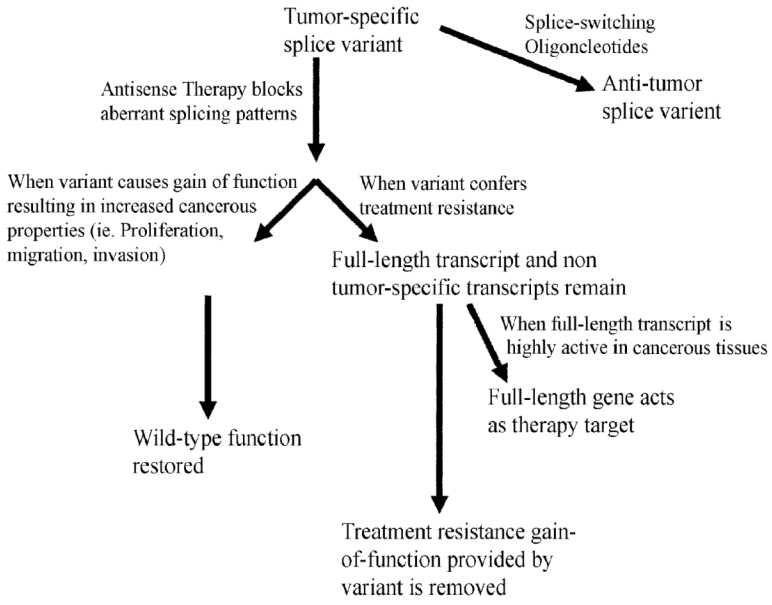

Fig. 2.
Application of treatment strategies